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ORIGINAL REPORT

Phase II Study of Everolimus and Letrozole in Patients With Recurrent Endometrial Carcinoma

Brian M. Slomovitz, Yunyun Jiang, Melinda S. Yates, Pamela T. Soliman, Taren Johnston, Maureen Nowakowski, Charles Levenback, Qian Zhang, Kari Ring, Mark F. Munsell, David M. Gershenson, Karen H. Lu, and Robert L. Coleman

Brian M. Slomovitz, Yunyun Jiang, Melinda S. Yates, Pamela T. Soliman, Taren Johnston, Charles Levenback, Qian Zhang, Kari Ring, Mark F. Munsell, David M. Gershenson, Karen H. Lu, and Robert L. Coleman, The University of Texas MD Anderson Cancer Center, Houston, TX; Brian M. Slomovitz and Maureen Nowakowski, Morristown Medical Center, Morristown, NJ; and Brian M. Slomovitz, Sylvester Comprehensive Cancer Center, University of Miami, Miller School of Medicine, Miami, FL.

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Corresponding author: Robert L. Coleman, MD, The University of Texas MD Anderson Cancer Center, 1155 Herman Pressler Dr, Unit No. 1362, Room No. CPB6.3244, Houston, TX 77030; e-mail: rcoleman@mdanderson.org.

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ABSTRACT

Purpose
The phosphoinositol-3 kinase (PI3K) pathway is frequently dysregulated in endometrial cancer (EC). Hormonal manipulation leads to response in some patients with EC, but resistance derived from PI3K pathway activation has been documented. Targeting mammalian target of rapamycin (mTOR) may overcome endocrine resistance. We conducted a two-institution phase II trial of everolimus and letrozole in women with recurrent EC.

Patients and Methods
Patients were considered incurable, had measurable disease, and were treated with up to two prior cytotoxic regimens. Everolimus was administered orally at 10 mg daily and letrozole was administered orally at 2.5 mg daily. Each cycle consisted of 4 weeks of therapy. Patients were treated until progression, toxicity, or complete response (CR). The primary end point was the clinical benefit rate (CBR), which was defined as CR, partial response, or stable disease (≥ 16 weeks) by RECIST 1.0 criteria. Translational studies were performed to correlate biomarkers with response.

Results
Thirty-eight patients were enrolled (median age, 62 years; range, 24 to 82 years). Thirty-five patients were evaluable for response. The CBR was 40% (14 of 35 patients); the median number of cycles among responders was 15 (range, seven to 29 cycles). The confirmed objective response rate (RR) was 32% (11 of 35 patients; nine CRs and two partial responses; median, 15 cycles; range, eight to 29 cycles). Twenty percent of patients (seven of 35 patients) were taken off treatment after a prolonged CR and at the discretion of the treating clinician. None of the patients discontinued treatment as a result of toxicity. Serous histology was the best predictor of lack of response. Patients with endometrioid histology and *CTNNB1* mutations responded well to everolimus and letrozole.

Conclusion
Everolimus plus letrozole results in a high CBR and RR in patients with recurrent EC. Further development of this combination in recurrent endometrioid EC is under way.

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INTRODUCTION

In the United States, endometrial cancer (EC) remains the most commonly diagnosed gynecologic malignancy. The majority of women with EC will be cured with surgery alone or in combination with adjuvant therapy; however, more than 8,000 women die annually, predominately as a result of resistance to conventional therapy. Recent molecular profiling has shown that increased phosphoinositol-3 kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling is associated with aggressive disease and poor prognosis.[1]

In patients with recurrent and/or metastatic EC, single-agent treatment with the mTOR inhibitors everolimus, temsirolimus, and ridaforolimus has led to clinical benefit rates (CBRs) of 21%,[2] 52% to 83%,[3] and 33% to 66%,[4,5] respectively. In a randomized phase II trial, ridaforolimus was associated with a significantly longer progression-free survival (PFS) compared with hormonal therapy or chemotherapy.[6] The toxicity profile of mTOR inhibitors is favorable. One common adverse effect, hyperglycemia, is a possible on-target effect of PI3K/AKT/mTOR pathway inhibition.[2,6]

There is a long history of studying hormonal therapy in women with advanced or recurrent EC.

Although such regimens are well tolerated and may produce responses of long duration in selected patients, the overall response rates (RRs) and PFS have been disappointing.

Given the well-documented importance of estrogen receptor (ER) signaling as a driver of type I EC[7] and cross-regulation between the ER and PI3K/AKT/mTOR pathways,[8] synergistic antitumor effects might be achieved by combining PI3K/AKT/mTOR pathway inhibitors with agents that disrupt ER signaling. We hypothesized that mTOR inhibition in combination with hormonal therapy may have an additive or synergistic effect and improve the RR over either agent alone. The combination of everolimus with the aromatase inhibitor, exemestane, significantly improved PFS in patients with aromatase inhibitor–refractory breast cancer,[9] thus demonstrating proof of concept that PI3K/AKT/mTOR pathway inhibitors may reverse resistance to endocrine therapy.

Herein, we report, to our knowledge, the first comprehensive phase II trial of mTOR inhibition in combination with hormonal therapy for the treatment of recurrent, pretreated EC. We demonstrate clinical activity not previously observed among similar patients treated with either agent alone.

PATIENTS AND METHODS

We designed and conducted a phase II, open-label trial at The University of Texas MD Anderson Cancer Center and Morristown Medical Center (Atlantic Health Systems, Morristown, NJ). The primary objective of this study was to determine the efficacy of everolimus (provided by Novartis, Basel, Switzerland) in combination with letrozole (provided by Novartis) in patients with recurrent or progressive EC. We also sought to evaluate toxicity, duration of disease control, time to disease progression, and survival in this cohort of patients. Molecular biomarkers were evaluated for correlation with response to therapy, overall survival (OS), and PFS. This study is registered on the clinical trial Web site of the National Cancer Institute (ClinicalTrials.gov identifier: NCT01068249). Institutional review board approval from both institutions was obtained.

Patient Population

Patients with progressive or recurrent EC who had received up to two prior chemotherapeutic regimens were eligible for this investigator-initiated phase II trial. Eligible patients were required to have histologically confirmed EC. Patients with carcinosarcoma or sarcoma were excluded. Patients were required to have a Zubrod performance status of 0 to 2 and no history of an invasive malignancy other than EC within a 5-year period before trial entry. Patients were required to have measurable disease by RECIST 1.0.[10]

Pretreatment hematologic, renal, and hepatic function tests were required to be grade 0 or 1 according to Common Terminology Criteria for Adverse Events (version 3.0). Other protocol details are available in the protocol (Data Supplement).

Treatment Plan and Response Evaluation

All patients were treated on an outpatient basis and were treated until disease progression, dose-limiting toxicity, or confirmed complete response (CR). For patients with a prolonged CR, the decision to stop therapy was made at the discretion of the treating physician. The initial dose of everolimus was 10 mg daily, and the dose of letrozole was 2.5 mg daily; both drugs were given orally. Registration of all patients was managed at the Investigational New Drug office at The University of Texas MD Anderson Cancer Center.

Each cycle consisted of 4 weeks of therapy. Records of study medication used, doses administered, and intervals between visits were kept during the study. The primary efficacy end point was CBR, defined as prolonged stable disease (SD; $\geq$ 16 weeks) or CR or partial response by RECIST criteria. Imaging was conducted at 8 and 16 weeks from first dosing regardless of treatment interruptions. Subsequent imaging was performed every 12 weeks thereafter, unless there was obvious progressive disease (PD) by physical examination, clinical deterioration, or new symptomatology suggestive of clinical disease progression. Best response was confirmed by subsequent imaging, and duration of response was measured from confirmation until PD. Patients discontinuing therapy in the absence of progression or toxicity were censored at last known follow-up. Patients with subsequent imaging and those in whom treatment was discontinued for toxicity were considered uncensored events.

Toxicity was assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). General guidelines for dose modifications in the event of hematologic and nonhematologic toxicities are available in the protocol.

Increased lipid levels are a known adverse effect of treatment with both everolimus and aromatase inhibitors. In case of hyperlipidemia ($\geq$ grade 1 hypercholesterolemia with or without hypertriglyceridemia), in addition to dietary advice to patients, a hydroxymethylglutaryl-coenzyme A reductase inhibitor was allowed with study treatment. Any grade of hyperglycemia was managed with medical therapy, most commonly metformin. Grade 3 hyperglycemia was managed with a dose reduction, and grade 4 was managed by discontinuing everolimus.

Correlative Studies

Immunohistochemistry. Formalin-fixed paraffin-embedded (FFPE) sections were stained using primary antibodies against ER (ER clone 6F11; Leica Biosystems, Buffalo Grove, IL) and progesterone receptor (PgR; PgR 1294; Dako, Carpinteria, CA) and independently scored by two reviewers. Nuclear staining was scored based on previously published methods.[11] Briefly, the proportions of positively stained tumor cells were assigned scores from 0 to 5 (0, none; 1, $<$ 1/100; 2, 1/100 to 1/10; 3, 1/10 to 1/3; 4, 1/3 to 2/3; and 5, $>$ 2/3). Then, the average intensities of positively stained tumor cells were assigned scores from 0 to 3 (0, none; 1, weak; 2, intermediate; and 3, strong). The sum of the proportion and intensity scores is the total score, ranging from 0 to 8. Tumor samples with a total score greater than 3 are considered positively stained. In the case of disagreement, a third reviewer would have been consulted and the case reviewed for consensus.

Mutational analysis. FFPE tumor tissue from primary hysterectomy specimens was used for DNA extraction by the QIAamp DNA FFPE Tissue Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). Mutational analysis of *KRAS* (codons 12 and 13 in exon 12; exons 3 to 5), *PIK3CA* (exons 9 and 20), and *CTNNB1* (exon 3) was performed using polymerase chain reaction–based Sanger sequencing at The University of Texas MD Anderson Cancer Center Sequencing and Microarray Core Facility. The primers used and areas covered are listed in Appendix Table A1 (online only).

Statistical Design

This phase II activity study incorporated a Bayesian design with multiple early stopping points. The target CBR rate was 20%. This was based on our previous study of single-agent everolimus in patients with pretreated, recurrent EC, which had a 21% confirmed CBR at 20 weeks.[2] The design was to accrue a minimum of 10 patients and a maximum of 35 patients. CBR was evaluated as patients were accrued, and the trial was to be stopped if there was evidence that the target CBR rate could not be met (ie, given the outcomes of the patients who had already been evaluated, if we determined that there was a $<$ 10% chance that the CBR rate was $<$ 20%). This decision rule gives the following stopping rule. We assume a uniform prior distribution for the rate of objective response or SD. Stop the trial if less than one of 10, two of 17, three of 24, or four of 31 patients evaluated had an objective response or SD. The operating characteristics of this study design are listed in [Appendix Table A2 (online only).

Statistical Analysis

Descriptive statistics were used to summarize the demographic and clinical characteristics of patients. We used Fisher's exact test to compare best response and clinical benefit between categories of histology and *KRAS*, *PI3KCA*, and *CTNNB1* mutation status. We used the Kaplan-Meier product-limit estimator[12] to estimate OS and PFS from the start of therapy, and we used

Table 1. Clinical Characteristics of Enrolled Patients

Characteristic	No. of Patients (N = 38)	%
Age, years		
Median	62	
Range	24-82	
Body mass index, kg/m^2		
Median (range)	28.6	
Range	20.8-46.7	
Stage of disease		
I	12	32
II	2	5
III	10	26
IV	9	24
Unknown	5	13
Grade		
1	6	16
2	17	45
3	15	39
Histology		
Endometrioid	27	71
Serous/clear cell/mixed	11	29
No. of prior chemotherapy regimens		
0	3	8
1	20	53
2	15	39

Table 2. Clinical Outcome

Outcome	No. of Patients	%
Clinical benefit		
No	21	60.0
Yes	14	40.0
Best response		
Complete response	9	25.7
Partial response	2	5.7
Stable disease	3	8.6
Progressive disease	21	60.0
Reason off study		
Completed treatment	7	20.0
Progressive disease	28	80.0
Progressive disease		
No	4	11.4
Yes	31	88.6
Current status		
Alive with disease	5	14.3
No evidence of disease	4	11.4
Dead	26	74.3
Follow-up, months		
Mean	18.1	
Standard deviation	14.5	
Median	14	
Range	1.4-46.8	

Cox proportional hazards regression[13] to model OS and PFS as functions of potential prognostic factors, such as histology and *KRAS*, *PI3KCA*, and *CTNNB1* mutation status. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

RESULTS

Patients

A total of 38 patients were enrolled onto the trial between May 2010 and September 2011. The median patient age was 62 years (range, 24 to 82 years). Study patients received a total of 264 cycles of therapy. All 38 women were evaluable for toxicity; 35 were evaluable for response. Three patients were not evaluable after receiving less than one cycle of therapy (rapid deterioration, n = 2; noncompliance, n = 1). Of the evaluable patients, 94% received prior chemotherapy for recurrent disease (63% received one prior regimen and 31% received two prior regimens). Forty-three percent of patients received prior radiotherapy. Baseline characteristics of the enrolled patients are listed in Table 1.

Six study participants were diabetic at the time of enrollment; none of these patients were insulin dependent. Hemoglobin A1c measurement within 3 months before study enrollment was available for only two of six patients (hemoglobin A1c values of 5.5% and 5.8%).

Efficacy

Of the 35 evaluable patients, the confirmed CBR at 16 weeks was 40% (14 patients; Table 2). The median number of cycles among responders was 15 (range, seven to 29 cycles). The objective confirmed RR was 32% (95% CI, 17% to 49%; 11 of 35 patients) and included nine patients with a CR. All patients have completed treatment. Twenty percent of patients (seven of 35 patients) were taken off of therapy at the discretion of their clinician after a prolonged CR. Median follow-up time for all patients was 14 months (range, 1.4 to 46.8 months), and median follow-up time for the nine patients alive at last contact was 40.8 months (range, 28.7 to 46.8 months).

Twenty-six patients have died. Median OS time was 14 months (95% CI, 9.5 to 24.4 months). OS is shown in Appendix Figure A1 (online only). The 6-month OS rate was 71.4% (95% CI, 57.9% to 88.1%). The 12-month OS rate was 54.3% (95% CI, 40.1% to 73.6%).

Four patients did not have PD, and all four of these patients remain alive. Thirty-one patients had PD, and five of these patients remain alive. Median PFS time was 3.0 months (95% CI, 1.9 to 15.7 months). The 6-month PFS rate for this cohort was 42% (95% CI, 29.2% to 62.8%). The 12-month PFS rate was 37.1% (95% CI, 24.1% to 57.2%; Fig 1).

Safety

The safety population of 38 patients included all patients who received at least one dose of the study drugs. Twelve patients required a dose reduction of everolimus to 5 mg daily because of stomatitis (n = 3), hyperglycemia (n = 4), thrombocytopenia (n = 2), elevated liver function tests (n = 1), infection (n = 1), and nausea (n = 1). The most common adverse events considered possibly, probably, or definitely related to the combination of everolimus and letrozole were fatigue (74%), hypertriglyceridemia (74%), hypercholesterolemia (71%), mucositis (66%), anemia (61%), hyperglycemia (55%), and nausea (53%; Appendix Table A3, online only). Most events were grade 1 or 2 toxicities and easily managed. None of the patients discontinued therapy as a result of toxicity.

Of interest, concomitant use of metformin for treatment of either pre-existing or protocol-related hyperglycemia occurred in

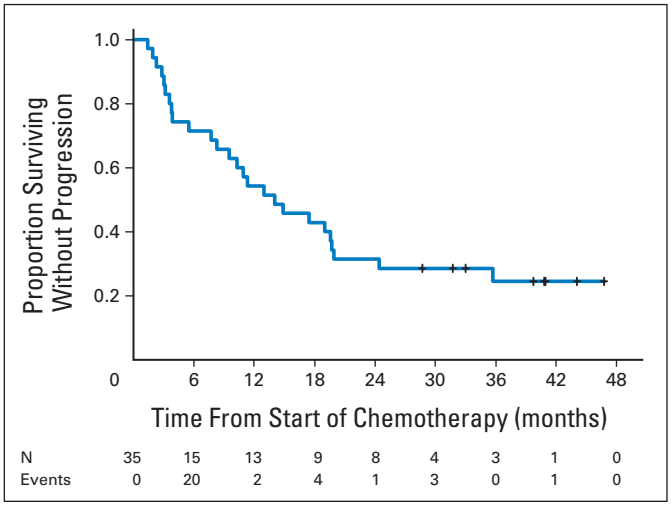

Fig 1. Progression-free survival.

nine patients. The RR for metformin users was 56% (*v* 23% for nonusers; $P < .05$).

ER/PgR Status

Of the 35 evaluable patients, 30 patients (including 23 patients with endometrioid EC) had additional tissue sections or clinical pathology results to determine ER/PgR status by immunohistochemistry. All seven serous cases had PD, including two patients with positive ER/PgR staining and five patients with negative ER/PgR staining. Of the 23 patients with endometrioid EC, 65% (13 of 20 patients) with positive ER staining and 33% (one of three patients) with negative ER staining had clinical benefit ($P = .54$). In addition, of the 23 patients with endometrioid EC, 68% (13 of 19 patients) with positive PgR staining and 25% (one of four patients) with negative PgR staining had an objective response or SD ($P = .26$).

Mutational Analysis

Of the 35 evaluable patients, 25 had DNA available for mutational analysis. Among them, three patients (12%) had *KRAS* mutations, five patients (20%) had *PIK3CA* mutations, and five patients (20%) had *CTNNB1* mutations. All identified mutations are listed in Appendix Table A4 (online only).

Correlative studies, including mutation analysis, as potential prognostic factors for CBR are listed in Table 3. OS and PFS for several potential prognostic factors are listed in Tables 4.

DISCUSSION

Letrozole in combination with everolimus showed a high CBR, high RR, and overall better than expected activity in a phase II clinical trial in advanced EC. The safety profile of everolimus was acceptable in the context of pretreated patients with EC.

The strongest predictor of nonresponse to everolimus/letrozole therapy is serous histology. Other single-agent studies of rapalogs have seen similar findings with few patients with serous histology responding to mTOR inhibition.[2,4-6,14] Because of the different pathogenesis of serous cancers and differing serous-like molecular profile defined by The Cancer Genome Atlas uterine analysis, a different treatment response may not be surprising. Further analysis of patients with endometrioid tumors suggests heterogeneity even within one subtype. A single group of responders is difficult to define in patients with endometrioid EC in this study, but there are hints at multiple molecular subgroups that may benefit from everolimus/letrozole treatment. Additional studies are essential to clearly define these subgroups. For example, many women with low-grade, ER-/PgR-positive disease had an objective response or SD (similar to what might traditionally be predicted for hormone therapy), yet a surprisingly large portion of women with this same tumor subtype had PD. A recent study by Liu et al[16] analyzed comprehensive molecular profiling from The Cancer Genome Atlas and identified distinct molecular subsets within the endometrioid endometrial subtype that were found to have different clinical outcomes. One molecular subtype is partially defined by the presence of *CTNNB1* mutations and is associated with worse outcomes compared with other endometrioid tumors. Our study suggests that patients with endometrioid EC with *CTNNB1* mutations may respond particularly well to everolimus/letrozole, and this therapy could be important for this aggressive subset of tumors. Although all patients with endometrioid EC with *CTNNB1* mutations (four of four patients) responded to treatment, a large portion of women with wild-type *CTNNB1* also responded (seven of 13 patients). Again, this hints that additional mutations, expression changes, or related pathway alterations may be contributing to treatment response. For example, *CTNNB1* encodes β-catenin, which binds to and is phosphorylated by GSK3β. This phosphorylation leads to degradation of β-catenin.[17]

In our study, mutations in *PIK3CA*, *KRAS*, and *CTNNB1* were detected in 22%, 11%, and 22% of patients, respectively. As a comparison, Makker et al[18] reported mutations in *PIK3CA*, *KRAS*, and *CTNNB1* in 24%, 28%, and 24% of patients with endometrioid tumors, respectively. Bourgon et al[19] reported a lower percentage of *CTNNB1* mutations (8%), but the lower observed prevalence is described as an artifact as a result of the incomplete interrogation of the gene by their multiplexed polymerase chain reaction sequencing method. In addition, Bourgon et al[19] reported a higher percentage of *PIK3CA* mutations (58%), yet our results match closely with Makker et al[18] (22% *v* 24%, respectively).

Previous studies show that mutations in the binding region to GSK3β result in weakened interaction with GSK3β and stabilization of β-catenin.[20] Previous studies also suggest that stabilized β-catenin results in activation of mTORC1, which may lead to cell proliferation.[21] The addition of everolimus, which inhibits mTORC1, abrogates the activating effect of β-catenin. More comprehensive molecular profiling will be essential to identifying the most important drivers behind response (or nonresponse) to everolimus/letrozole therapy.

Treatment options for patients with recurrent EC are extremely limited, particularly for those in whom chemotherapy has been administered. A summary of agents studied in this context is presented in Table 5.[22-32] Our findings compare favorably to these historical controls, including single-agent paclitaxel and bevacizumab.

In our cohort, common adverse events included hyperglycemia (69%), hypercholesterolemia (49%), and hypertriglyceridemia (60%). The protocol allowed for these abnormalities to be corrected while on study. Nine patients took metformin during the study, including five patients who initiated therapy after study entry. The objective RR among metformin users was 56% (*v* 23% for nonusers; $P < .05$). Recently, the use of the hypoglycemic agent metformin was

Table 3. Response

	Histology							*KRAS* Mutation							*PI3KCA* Mutation							*CTNNB1* Mutation							Metformin						
	Endometrioid		Serous		Total			No		Yes		Total			No		Yes		Total			No		Yes		Total			No		Yes		Total		
Response	No. of Patients	%	No. of Patients	%	No. of Patients	%	*P*	No. of Patients	%	No. of Patients	%	No. of Patients	%	*P*	No. of Patients	%	No. of Patients	%	No. of Patients	%	*P*	No. of Patients	%	No. of Patients	%	No. of Patients	%	*P*	No. of Patients	%	No. of Patients	%	No. of Patients	%	*P*
Best response							.0092							1.000							.6574							.1779							.0932
CR	9	37.5	0	0.0	9	25.7		7	31.8	1	33.3	8	32.0		7	35.0	1	20.0	8	32.0		5	25.0	3	60.0	8	32.0		4	15.4	5	55.6	9	25.7	
PR	2	8.3	0	0.0	2	5.7		1	4.6	0	0.0	1	4.0		1	5.0	0	0.0	1	4.0		1	5.0	0	0.0	1	4.0		2	7.7	0	0.0	2	5.7	
SD	3	12.5	0	0.0	3	8.6		2	9.1	0	0.0	2	8.0		1	5.0	1	20.0	2	8.0		1	5.0	1	20.0	2	8.0		2	7.7	1	11.1	3	8.6	
PD	10	41.7	11	100.0	21	60.0		12	54.6	2	66.7	14	56.0		11	55.0	3	60.0	14	56.0		13	65.0	1	20.0	14	56.0		18	69.2	3	33.3	21	60.0	
Clinical benefit							< .001							1.000							1.000							.1333							.1122
No	10	41.7	11	100.0	21	60.0		12	54.6	2	66.7	14	56.0		11	55.0	3	60.0	14	56.0		13	65.0	1	20.0	14	56.0		18	69.2	3	33.3	21	60.0	
Yes	14	58.3	0	0.0	14	40.0		10	45.4	1	33.3	11	44.0		9	45.0	2	40.0	11	44.0		7	35.0	4	80.0	11	44.0		8	30.8	6	66.7	14	40.0	

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Table 4. Overall Survival and Progression-Free Survival

Factor	Overall Survival						Progression-Free Survival					
	No. of Patients	No. of Deaths	Median (months)	*P*	Hazard Ratio	95% CI	No. of Patients	No. of Events	Median (months)	*P*	Hazard Ratio	95% CI
Histology												
Endometrioid	24	15	19.8		Reference		24	20	12.3		Reference	
Serous	11	11	3.5	< .001	6.43	2.51 to 16.45	11	11	1.6	.0028	3.27	1.50 to 7.11
KRAS mutation												
No	22	15	15.7		Reference		22	19	8.2		Reference	
Yes	3	3	11.3	.4865	1.56	0.45 to 5.39	3	3	3.0	.2862	2.01	0.56 to 7.22
PI3KCA mutation												
No	20	15	12.1		Reference		20	18	6.0		Reference	
Yes	5	3	24.4	.3383	0.55	0.16 to 1.89	5	4	3.0	.7406	0.83	0.28 to 2.49
CTNNB1 mutation												
No	20	17	12.1		Reference		20	18	2.0		Reference	
Yes	5	1	NR	.0503	0.13	0.02 to 1.00	5	4	26.0	.0989	0.39	0.13 to 1.19
Metformin use												
No	26	21	11.6		Reference		26	24	1.9		Reference	
Yes	9	5	24.4	.1202	0.46	0.17 to 1.23	9	7	14.5	.1093	0.50	0.21 to 1.17

Abbreviation: NR, not reached.

shown to reduce the incidence of malignancies in patients with diabetes.[33,34] The clinical activity of metformin is now being investigated in several cancers, including EC. We have recently launched an open-label phase II activity trial evaluating everolimus, letrozole, and metformin in a similar group of patients (ClinicalTrials.gov identifier: NCT01797523).

Development of pneumonitis has been described in patients taking mTOR inhibitors.[35] In our study, three patients developed noninfectious pneumonitis; all three patients were treated conservatively by withholding therapy until the pneumonitis resolved. The everolimus was restarted in all patients without a dose reduction. All three patients had a positive response to therapy (two partial responses and one SD). In metastatic renal cell carcinoma, pneumonitis has been suggested as a biologic marker of response.[36] Further confirmation is needed to determine whether pneumonitis is also a marker of response in women with EC.

Table 5. GOG Experience With Recurrent Endometrial Cancer

Protocol	Agent	No. of Evaluable Patients	6-Month PFS (%)	No. of Patients Responding	Response Rate (%)
GOG 129-B	Etoposide[22]	25	8	0	0
GOG 129-C	Paclitaxel[23]	48	21	12	25
GOG 129-E	Dactinomycin[24]	27	4	3	11
GOG 129-H	Liposomal doxorubicin[25]	43	23	4	9
GOG 129-I	Pyrazoloacridine[26]	25	16	1	4
GOG 129-J	Topotecan[27]	28	25	2	7
GOG 129-K	Oxaliplatin[28]	52	27	7	13
GOG 129-L	Irofulven[29]	25	28	1	4
GOG 129-M	Flavopiridol[30]	21	0	0	0
GOG 229-B	Thalidomide[31]	24	8	3	12
GOG 229-E	Bevacizumab[32]	52	40	7	13.5
Current study	Everolimus/ letrozole	35	43	11	31

Abbreviations: GOG, Gynecologic Oncology Group; PFS, progression-free survival.

In addition to an encouraging CBR, the majority of complete responders had extended response duration. Of the nine patients who had a CR, four continue to have no evidence of disease 22 to 36 months after completion of the study regimen. Three patients remain off any treatment, and one patient has continued on letrozole and metformin off study. All four of these patients had ER-/PgR-positive tumors, but there were no other mutations identified in common among these responders. Although the remaining five patients eventually experienced progression, patients received 10 to 29 cycles of everolimus and letrozole, and only two of these patients experienced progression on study after 10 and 18 cycles, respectively. This extended response duration is similar to that seen in the Gynecologic Oncology Group (GOG) 153 trial with alternating courses of megestrol acetate and tamoxifen, which resulted in an overall RR of 27% and a response duration of at least 20 months in 53% of responders.[37] Given the comparable response and duration of response between the present trial and GOG-153, a randomized comparison of these two treatment strategies is planned (GOG-3007).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Brian M. Slomovitz, Mark F. Munsell, David M. Gershenson, Karen H. Lu, Robert L. Coleman
Administrative support: Robert L. Coleman
Provision of study materials or patients: Robert L. Coleman
Collection and assembly of data: Brian M. Slomovitz, Yunyun Jiang, Taren Johnston, Qian Zhang, Robert L. Coleman
Data analysis and interpretation: Brian M. Slomovitz, Yunyun Jiang, Melinda S. Yates, Pamela T. Soliman, Maureen Nowakowski, Charles

Levenback, Qian Zhang, Kari Ring, Mark F. Munsell, David M. Gershenson, Karen H. Lu, Robert L. Coleman

Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Catasus L, D'Angelo E, Pons C, et al: Expression profiling of 22 genes involved in the PI3K-AKT pathway identifies two subgroups of high-grade endometrial carcinomas with different molecular alterations. Mod Pathol 23:694-702, 2010

2. Slomovitz BM, Lu KH, Johnston T, et al: A phase 2 study of the oral mammalian target of rapamycin inhibitor, everolimus, in patients with recurrent endometrial carcinoma. Cancer 116:5415-5419, 2010

3. Abe K, Abe T, Adachi I, et al: Observation of chi(c2) production in B meson decay. Phys Rev Lett 89:011803, 2002

4. Colombo N, McMeekin DS, Schwartz PE, et al: Ridaforolimus as a single agent in advanced endometrial cancer: Results of a single-arm, phase 2 trial. Br J Cancer 108:1021-1026, 2013

5. Mackay H, Welch S, Tsao MS, et al: Phase II study of oral ridaforolimus in patients with metastatic and/or locally advanced recurrent endometrial cancer. J Clin Oncol 29, 2011 (suppl; abstr 5013)

6. Oza AM, Poveda A, Clamp AR, et al: A randomized phase II (RP2) trial of ridaforolimus (R) compared with progestin (P) or chemotherapy (C) in female adult patients with advanced endometrial carcinoma. J Clin Oncol 29, 2011 (suppl; abstr 5009)

7. Bokhman JV: Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 15:10-17, 1983

8. Cheung LW, Hennessy BT, Li J, et al: High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. Cancer Discov 1:170-185, 2011

9. Baselga J, Campone M, Piccart M, et al: Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 366:520-529, 2012

10. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000

11. Harvey JM, Clark GM, Osborne CK, et al: Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 17:1474-1481, 1999

12. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

13. Cox DR: Regression models and life tables. J R Stat Soc B 34:187-220, 1972

14. Fleming GF, Filiaci VL, Marzullo B, et al: Temsirolimus with or without megestrol acetate and tamoxifen for endometrial cancer: A Gynecologic Oncology Group study. Gynecol Oncol 132:585-592, 2014

15. Reference deleted

16. Liu Y, Patel L, Mills GB, et al: Clinical significance of CTNNB1 mutation and Wnt pathway activation in endometrioid endometrial carcinoma. J Natl Cancer Inst [epub ahead of print on September 10, 2014

17. Anastas JN, Moon RT: WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer 13:11-26, 2013

18. Makker V, Recio FO, Ma L, et al: Phase II trial of GDC-0980 (dual PI3K/mTOR inhibitor) in patients with advanced endometrial carcinoma: Final study results. J Clin Oncol 32, 2014 (suppl 5s; abstr 5513)

19. Bourgon R, Lu S, Yan Y, et al: High-throughput detection of clinically relevant mutations in archived tumor samples by multiplexed PCR and next-generation sequencing. Clin Cancer Res 20:2080-2091, 2014

20. Morin PJ, Sparks AB, Korinek V, et al: Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275:1787-1790, 1997

21. Fujishita T, Aoki K, Lane HA, et al: Inhibition of the mTORC1 pathway suppresses intestinal polyp formation and reduces mortality in ApcDelta716 mice. Proc Natl Acad Sci U S A 105:13544-13549, 2008

22. Slayton RE, Blessing JA, Delgado G: Phase II trial of etoposide in the management of advanced or recurrent endometrial carcinoma: A Gynecologic Oncology Group study. Cancer Treat Rep 66:1669-1671, 1982

23. Lincoln S, Blessing JA, Lee RB, et al: Activity of paclitaxel as second-line chemotherapy in endometrial carcinoma: A Gynecologic Oncology Group study. Gynecol Oncol 88:277-281, 2003

24. Moore DH, Blessing JA, Dunton C, et al: Dactinomycin in the treatment of recurrent or persistent endometrial carcinoma: A phase II study of the Gynecologic Oncology Group. Gynecol Oncol 75:473-475, 1999

25. Homesley HD, Blessing JA, Sorosky J, et al: Phase II trial of liposomal doxorubicin at 40 mg/m(2) every 4 weeks in endometrial carcinoma: A Gynecologic Oncology Group study. Gynecol Oncol 98:294-298, 2005

26. Plaxe SC, Blessing JA, Husseinzadeh N, et al: Phase II trial of pyrazoloacridine in patients with persistent or recurrent endometrial carcinoma: A Gynecologic Oncology Group study. Gynecol Oncol 84:241-244, 2002

27. Miller DS, Blessing JA, Lentz SS, et al: A phase II trial of topotecan in patients with advanced, persistent, or recurrent endometrial carcinoma: A Gynecologic Oncology Group study. Gynecol Oncol 87:247-251, 2002

28. Fracasso PM, Blessing JA, Molpus KL, et al: Phase II study of oxaliplatin as second-line chemotherapy in endometrial carcinoma: A Gynecologic Oncology Group study. Gynecol Oncol 103:523-526, 2006

29. Schilder RJ, Blessing JA, Pearl ML, et al: Evaluation of irofulven (MGI-114) in the treatment of recurrent or persistent endometrial carcinoma: A phase II study of the Gynecologic Oncology Group. Invest New Drugs 22:343-349, 2004

30. Grendys EC Jr, Blessing JA, Burger R, et al: A phase II evaluation of flavopiridol as second-line chemotherapy of endometrial carcinoma: A Gynecologic Oncology Group study. Gynecol Oncol 98:249-253, 2005

31. McMeekin DS, Sill MW, Benbrook D, et al: A phase II trial of thalidomide in patients with refractory endometrial cancer and correlation with angiogenesis biomarkers: A Gynecologic Oncology Group study. Gynecol Oncol 105:508-516, 2007

32. Aghajanian C, Sill MW, Darcy KM, et al: Phase II trial of bevacizumab in recurrent or persistent endometrial cancer: A Gynecologic Oncology Group study. J Clin Oncol 29:2259-2265, 2011

33. Yin M, Zhou J, Gorak EJ, et al: Metformin is associated with survival benefit in cancer patients with concurrent type 2 diabetes: A systematic review and meta-analysis. Oncologist 18:1248-1255, 2013

34. Thompson AM: Molecular pathways: Preclinical models and clinical trials with metformin in breast cancer. Clin Cancer Res 20:2508-2515, 2014

35. Duran I, Goebell PJ, Papazisis K, et al: Drug-induced pneumonitis in cancer patients treated with mTOR inhibitors: Management and insights into possible mechanisms. Expert Opin Drug Saf 13:361-372, 2014

36. Atkinson BJ, Cauley DH, Ng C, et al: Mammalian target of rapamycin (mTOR) inhibitor-associated non-infectious pneumonitis in patients with renal cell cancer: Predictors, management, and outcomes. BJU Int 113:376-382, 2014

37. Fiorica JV, Brunetto VL, Hanjani P, et al: Phase II trial of alternating courses of megestrol acetate and tamoxifen in advanced endometrial carcinoma: A Gynecologic Oncology Group study. Gynecol Oncol 92:10-14, 2004

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Phase II Study of Everolimus and Letrozole in Patients With Recurrent Endometrial Carcinoma

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Kari Ring
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Appendix

Table A1. List of Primers Used for Sequencing

Primer Name	Targeting Region	Sequence
KRAS-2-5′	*KRAS* 12, 13	TAAGGCCTGCTGAAAATGACTG*
KRAS-2-3′	*KRAS* 12, 13	TGGTCCTGCACCAGTAATATGC*
KRAS-3(+)	*KRAS* exon 3	CTGTGTTTCTCCCTTCTC
KRAS-3(–)	*KRAS* exon 3	CATGGCATTAGCAAAGACTC
KRAS-4(+)	*KRAS* exon 4	GGTGTAGTGGAAACTAGG
KRAS-4(–)	*KRAS* exon 4	GCAATGCCCTCTCAAGAG
KRAS-5(+)	*KRAS* exon 5	CTTGCACATGGCTTTCCCAG
KRAS-5(–)	*KRAS* exon 5	GTTGCCACCTTGTTACC
PIK3CA-9(+)	*PIK3CA* exon 9	CATCTGTGAATCCAGAGG
PIK3CA-9(–)	*PIK3CA* exon 9	CTGAGATCAGCCAAATTC
PIK3CA-20(+)	*PIK3CA* exon 20	GCTTTGTCTACGAAAGCC
PIk3CA-20(–)	*PIK3CA* exon 20	GGAATCCAGAGTGAGC
CTNNB1-3(+)	*CTNNB1* exon 3	CAATGGGTCATATCACAG
CTNNB1-3(–)	*CTNNB1* exon 3	CTGACTTTCAGTAAGGCAATG

*Ho CL, et al: Cancer Res 64:6915-6918, 2004.

Table A2. Decision Rule for Stopping the Trial

Rate of CR + PR + SD	Probability of Stopping Early
0.05	0.954
0.10	0.725
0.15	0.434
0.20	0.215
0.25	0.106

Abbreviations: CR, complete response; PR, partial response; SD, stable disease.

Table A3. Adverse Events Considered Possibly, Probably, or Definitely Related to Everolimus/Letrozole

	All Grades		Grade 3 to 4	
Adverse Event	No. of Patients	%	No. of Patients	%
Abdominal pain	5	13	0	0
ALT increased	14	37	2	5
Alkaline phosphatase increased	1	3	0	0
Allergic rhinitis	3	8	0	0
Allergy/immunology (other)	2	5	0	0
Alopecia	2	5	0	0
Anorexia	16	42	0	0
Anxiety	1	3	0	0
AST increased	2	5	0	0
Auditory/ear (other)	1	3	0	0
Back pain	1	3	0	0
Bilirubin increased	2	5	0	0
Bladder pain	1	3	0	0
Blood glucose increased	21	55	2	5
Blood/bone marrow (other)	1	3	0	0
Bruising	1	3	0	0
Cardiac arrhythmia (other)	1	3	0	0
Chills	5	13	0	0
Confusion	1	3	0	0
Constipation	3	8	0	0
Constitutional symptoms (other)	2	5	0	0
Cough	13	34	0	0
Creatinine increased	4	11	0	0
Dehydration	1	3	0	0
Dermatology/skin (other)	1	3	0	0
Diarrhea	12	32	2	5
Dizziness	5	13	0	0
Dry eye syndrome	2	5	0	0
Dry mouth	5	13	1	3
Dry skin	6	16	0	0
Dysphagia	1	3	0	0
Dyspnea	8	21	1	3
Edema limbs	11	29	0	0
Endocrine (other)	1	3	0	0
Enteritis	1	3	1	3
Fatigue	28	74	4	11
Fever	5	13	0	0
Flatulence	2	5	0	0
Flushing	2	5	0	0
GI (other)	1	3	0	0
Headache	15	39	2	5
Hemoglobin decreased	23	61	2	5
Hemorrhage nasal	5	13	0	0
Hot flashes	2	5	0	0
Hypertension	2	5	1	3
Infection	1	3	0	0
Infection (other)	1	3	0	0
Insomnia	5	13	1	3
Joint pain	1	5	0	0
Leukopenia	13	34	0	0
Lymphopenia	2	5	0	0
Memory impairment	2	5	0	0
Metabolic/laboratory (other)	11	29	0	0
Mucositis oral	25	66	1	3
Muscle weakness	1	3	0	0
Musculoskeletal (other)	2	5	1	3
Myalgia	5	13	1	3

(continued on following page)

Table A3. Adverse Events Considered Possibly, Probably, or Definitely Related to Everolimus/Letrozole (continued)

Adverse Event	All Grades		Grade 3 to 4	
	No. of Patients	%	No. of Patients	%
Nail disorder	6	16	0	0
Nasal congestion	1	3	0	0
Nausea	20	53	1	3
Neutrophil count decreased	5	13	0	0
Oral pain	4	11	0	0
Pain	4	11	0	0
Pain (other)	5	13	1	3
Pain in extremity	7	18	1	3
Paranasal sinus infection	1	3	1	3
Peripheral sensory neuropathy	12	32	1	3
Pharyngolaryngeal pain	1	3	1	3
Platelet count decreased	11	29	2	5
Pneumonia	3	8	1	3
Pneumonitis	3	8	0	0
Pruritus	9	24	0	0
Pulmonary (other)	3	8	0	0
Pulmonary hemorrhage	1	3	0	0
Rash acneiform	14	37	0	0
Rash desquamating	3	8	0	0
Serum cholesterol increased	27	71	1	3
Serum glucose decreased	1	3	0	0
Serum magnesium decreased	13	34	0	0
Serum potassium increased	1	3	0	0
Serum sodium decreased	3	8	0	0
Serum sodium increased	2	5	0	0
Serum triglyceride increased	28	74	2	5
Serum potassium decreased	11	29	1	3
Sinus tachycardia	1	3	0	0
Sinusitis	1	3	0	0
Soft tissue infection	2	5	0	0
Stomal ulcer	1	3	0	0
Sweating	7	18	0	0
Taste alteration	9	24	0	0
Tinnitus	2	5	0	0
Urinary frequency	3	8	0	0
Urinary tract infection	5	13	0	0
Vomiting	10	26	1	3
Watering eyes	1	3	0	0
Weight loss	8	21	0	0

Table A4. Complete List of Mutations

Acc	Mutation	Response	Histology
1	*CTNNB1* D32V	CR	Endometrioid
6	ND	CR	Endometrioid
9	ND	CR	Endometrioid
18	*CTNNB1* H36Y&S37C	CR	Endometrioid
25	ND	CR	Endometrioid
27	*CTNNB1* S37Y	CR	Endometrioid
32	*PIK3CA* D1045N	CR	Endometrioid
34	*KRAS* G12D	CR	Endometrioid
43	ND	PR	Endometrioid
17	*PIK3CA* E545D and *CTNNB1* T75I*	SD	Endometrioid
31	ND	SD	Endometrioid
2	ND	PD	Endometrioid
3	*KRAS* G12V	PD	Serous
4	ND	PD	Serous
7	ND	PD	Endometrioid
10	*CTNNB1* A20V&V22I	PD	Serous
14	ND	PD	Endometrioid
20	ND	PD	Serous
23	ND	PD	Endometrioid
28	ND	PD	Mixed
30	ND	PD	Serous/clear cell
36	*KRAS* P121S* and *PIK3CA* H1047R	PD	Endometrioid
37	*PIK3CA* E542K	PD	Endometrioid
41	ND	PD	Serous/clear cell
42	*PIK3CA* E545K	PD	Serous

Abbreviations: Acc, accession number; CR, complete response; ND, not done; PD, progressive disease; PR, partial response; SD, stable disease.
*Novel mutations.

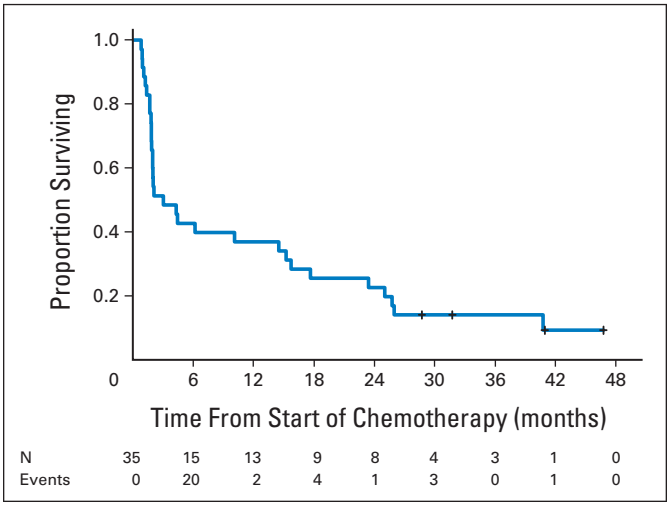

Fig A1. Overall survival.